CONTRIBUTION TO THE LIFE HISTORY OF SAGITTARIA VARIABILIS.[1]

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(WITH PLATES XX–XXVI)

THE following work upon Sagittaria was begun in October 1896, and is a continuation of my former work on *Alisma Plantago*,[2] to which paper frequent reference will be made for comparison. So far as the writer knows, no special work has been done upon the gametophyte generation of Sagittaria, or upon its embryology. The material used was killed in a solution of chrom-acetic acid and preserved in 70 per cent. alcohol, and the usual methods of imbedding in paraffin and staining on the slide were employed. The stain used for the greater part of the work was a double stain of anilin-safranin and gentian-violet.

The investigation was carried on under the direction of Dr. John M. Coulter, to whom I here express my thanks for assistance and criticism.

The flowers of *Sagittaria variabilis* are all monosporangiate, but frequently there are abortive carpels at the center of the staminate flower. Some varieties are monœcious and others diœcious. The carpels, which become achenes, are spirally arranged upon a very globose receptacle, as are also the stamens. The ovules are apotropous. In the earlier stages they are anatropous, but later they become strongly campylotropous, so that the mature embryo is bent double and becomes horseshoe shaped. Both the staminate and carpellate flowers have nectaries which are active during the blooming period. The nectaries are situated around the base of the flower, between the carpels and the petals. They appear to be simply modified

[1] Contributions from the Hull Botanical Laboratory. IV.

[2] The embryo sac of Alisma Plantago. BOT. GAZ. **21**: 123–132. 1896.

carpels. The glandular secreting cells are epidermal and are situated around the lower part (*fig. 1*), usually extending to the adjoining carpels, which often remain sterile and develop no embryo. The secreting cells begin to enlarge about the time the embryo sac is formed, and after fertilization is accomplished they cease their activity and become more or less shrunken and disorganized. During their active period the cytoplasm of these cells has the characteristic glandular appearance, and the nuclei are drawn out into irregular shapes, often having thick projections like pseudopodia (*fig. 45*).

DEVELOPMENT OF THE MALE GAMETOPHYTE.

As no suitable material was available the early development of the anther was not studied. The pollen mother cells were found dividing abundantly. In these numerous figures in the mother star stage showed large and well defined centrospheres at the poles (*fig. 2*), and although the exact number of chromosomes was not determined the reduction was ascertained to take place at this division. This fact should be kept in mind in connection with any theoretical explanation of the phenomenon of reduction, as it will be seen that the two daughter nuclei arising from the reduction nucleus do not belong to the first cells of the sexual generation, but to the mother cells of the microspores with which the sexual generation properly begins. By the time the nucleus of the pollen mother cells is in the close mother skein stage, two centrospheres appear at each pole of the spindle. By successive divisions the two microspore mother cells form the cells of the tetrad. These cells, which usually lie in one plane (*fig. 4*), soon separate, and with little or no increase in size develop into the microspores. The microspores possess a very thick wall, from whose outer surface are developed prickly projections (*fig. 5*).

The microspore soon begins to enlarge and the first division of its nucleus takes place, giving rise to the generative and tube nuclei. The two nuclei are at first quite similar, but they soon differentiate, the tube nucleus becoming larger, and the gener-

ative nucleus appearing to develop more chromatin, and thus always taking a deeper stain. Although no special staining was employed, centrospheres were frequently seen beside the resting generative nucleus. The pollen grain now rapidly grows to its mature size, and the generative nucleus immediately divides into the two sperm nuclei. These are small and spherical at first, and always stain so deeply that little or no structure can be seen in them (*fig. 7*). This division of the generative nucleus takes place long before the anther has reached its mature size and is ready to dehisce. After the pollen is shed the sperm nuclei no longer appear spherical, but are bean shaped or spindle shaped, and the tube nucleus shows a difference in its reaction, since it now stains almost as deeply as the sperm nuclei themselves, and shows little or none of its internal structure (*figs. 8, 9*). Whether the sperm nuclei organize definite cells I could not determine. The spindle shaped appearance may have been produced by the accumulation of a small amount of cytoplasm at the two ends, but if this was really the case my staining produced no differentiation between nucleus and cytoplasm. The division of the generative nucleus before pollination seems to be quite common in monocotyledons, and it is probable that this condition will be found to be the rule rather than the exception in this group.

DEVELOPMENT OF THE FEMALE GAMETOPHYTE.

Because of a lack of suitable material the development of the macrospore could not be worked out. The earliest stage found was a four-celled embryo sac (*fig. 10*). The two nuclei at the micropylar end arise by longitudinal division, while the two lower ones are produced by a transverse division. After the next divisions, which produce the typical eight-celled embryo sac, the nuclei begin to travel to their proper positions, while at the same time large vacuoles appear in their rear. The nuclei of the synergids, the nucleus of the oosphere, and the lower polar nucleus are about the same size, while the upper polar nucleus is by far the largest nucleus in the sac (*figs. 11, 12*). The three antipodal nuclei are considerably smaller than the others, and

even at this early stage, before the conjugation of the polar nuclei and the act of fertilization, they are often cut off by well defined cell walls (*fig. 11*).

In approaching each other the upper larger polar nucleus travels much farther than the lower one, so that the place of contact is usually in the lower part of the embryo sac (*figs. 14, 19, 25*), and the fusion takes place here without any apparent shifting of the nuclei, the fusion being usually complete before the entrance of the pollen tube into the sac (*fig. 19*). Frequently two centrosomes with a common hyaline area around them can be seen on one or both sides of the conjugating nuclei (*fig. 13*), indicating a possible union of the two pairs of centrospheres which are brought together when the two nuclei approach each other. Later the appearance is as though the two centrosomes had fused (*figs. 15, 16*). Although these observations were not very extensive, they agree with what I observed in the conjugating polar nuclei of *Alisma Plantago*. There is usually one large nucleolus in each polar nucleus, and during the fusion of the polar nuclei their nucleoli also seemed to fuse. When the nuclear fusion is nearly complete, two or three nucleoli appear close together (*figs. 15, 16*), and a little later the nucleoli are seen to lie in contact (*figs. 17, 18*). When nuclear fusion is completed, the definitive nucleus nearly always shows but one large nucleolus (*figs. 19, 29*), so there can be but little doubt from the stages observed that the nucleoli come together and fuse directly as definite bodies, without breaking up or being dissolved.

PHENOMENA OF FERTILIZATION.

Just before the entrance of the pollen tube into the micropyle, the two synergids lie side by side, with the oosphere suspended below and lying somewhat to one side (*figs. 14, 19, 20*). In the lower part of each synergid there is a large vacuole. At this stage the nucleus of the oosphere is usually quite symmetrical, being spherical or ellipsoidal in shape.

It will be remembered that the pollen grain has the two

sperm cells fully differentiated before pollination. As the tube passes through the micropyle it is considerably constricted, but when it reaches the apex of the embryo sac it increases appreciably in diameter. The tube takes exactly the same course as in Alisma, passing down on one side near the wall of the sac, and encountering the nucleus of one of the synergids, which disappears at this time and is never seen again (*figs. 21, 25, 26, 29*). The other synergid, with its nucleus, persists for a long time, and can still be seen above the vesicular suspensor cell of rather large embryos (*figs. 65, 69*). The pollen tube after entering the embryo sac stains very dark, and it is often difficult to distinguish the two sperm nuclei as they are traveling down the tube. In lightly stained material, however, they can be seen very readily. As the lower one approaches the tip of the tube it is preceded by two centrospheres, which can be seen always in well stained sections because of their position and the light colored cytoplasm with which they are usually surrounded (*figs. 22, 23, 24, 26, 28*). When the sperm nucleus breaks out of the tube it makes a decided perforation, the appearance being as though the tip had been softened and the nucleus had broken forcibly out of it. In some cases the edges of the perforation are rather smooth, while in other cases they are somewhat ragged (*figs. 29, 30, 31, 32*). A stream of cytoplasm escapes from the tube after the lower sperm nucleus (*fig. 30*), but the upper sperm nucleus never leaves the tube (*fig. 32*), which is also the case in Alisma. After the rupture of the pollen tube, the cytoplasm between the sexual cells usually contains numerous granules, which may have escaped from the tube, or they may be fragments of the disintegrated tip of the tube (*figs. 25, 27*). This often makes it difficult to identify the centrospheres at this stage, it being very easy to lose sight of them entirely although they may be present in the section.

In the meantime changes have been taking place in the oosphere. Its nucleus is no longer symmetrical in outline, but, just as in Alisma, it is drawn out into a considerable bulge on the side toward the sperm nucleus (*figs. 21, 24, 29, 30*). This

bulging of the female nucleus toward the male nucleus has also been observed in *Pinus Banksiana* and *P. Laricio*.[3] In the case of the large female nucleus of Pinus, however, the bulging appears only as a papilla-like protuberance, while in Alisma and Sagittaria the whole side of the nucleus appears to be drawn out. What the physiological significance of this bulging is cannot be stated, but it seems to be one of the characteristic phenomena of fertilization in the higher plants.

Although the method of staining employed did not bring out the centrospheres of the oosphere nucleus as readily as those of the sperm nucleus, they were sometimes seen, and when they appeared they were found lying just beyond the bulge of the oosphere nucleus toward the sperm nucleus (*fig. 21*). Thus, during the approach of the two sexual nuclei each one is preceded by its two centrospheres. Just before the contact of the sexual nuclei, two pairs of centrospheres appear on opposite sides of the approaching nuclei (*fig. 30*), and when the nuclei are in contact a little later, the two pairs appear to be fusing (*fig. 31*). These appearances are the same as those I observed during the fusion of the polar nuclei of Alisma, and seem to point strongly to a pairing and subsequent fusion of the four centrospheres which are thus brought together. Although these appearances very properly can receive such an explanation, it must be borne in mind that other movements and other explanations are possible. Thus, the two centrospheres which appear on the upper side in *figs. 30* and *31* may be interpreted as belonging to the female nucleus, while the lower pair may have come from the male. This would do away with the so-called "quadrille movement." I think, however, that Guignard's explanation of a conjugation in pairs is the more reasonable one, from the fact that during fusion of cells not only the nuclei themselves fuse, but apparently also the cytoplasm, chromatophores, and pyrenoids, indicating that during fusion all protoplasmic bodies of the same nature in the cells are involved in the act. The evidence which led me to infer a pairing of centrospheres during

[3] BOT. GAZ. 23: 40, 41. 1897.

the fusion of the polar nuclei in Alisma was the fact that just before the contact of the nuclei the centrospheres appeared a little farther apart as though they were separating. The interpretation in such cases, however, necessarily must be merely an inference, and could not be decided absolutely except by observing the phenomena in the living condition, which at present seems entirely out of the question in the case of the higher plants, unless a system of differential centrosphere stains can be developed. The whole subject must depend upon the question as to whether centrospheres are permanent bodies in the cell and deserve to rank with other cell organs. Although our knowledge in regard to these bodies is still too fragmentary to make any positive assertions, the permanent organ theory certainly seems to be receiving constantly new confirmation.. Recently Lauterborn[4] has studied these bodies in certain diatoms, in which he was able to see centrosomes very readily, even in the living condition. This is a direct confirmation of the work done by Bütschli,[5] who made the same observation in 1891. As far as my own observation goes, I can come to no other conclusion. These bodies appear so often that they can not be overlooked by a careful observer. They appear beside the resting nucleus, in the higher plants always two in number, and later at the poles of the spindle in the mother star stage. A little later, having divided into two, two centrospheres appear at each pole of the spindle, sometimes so prominent that they are as much in evidence as the nucleus itself (*fig. 34*). There is no reason why at times, especially during abnormal conditions, the centrospheres should not fragment or break up into a number of pieces, just as is the case with the nucleus, but any objections raised as to the individuality of centrospheres because of such action can have no more weight than a similar argument against the individuality of the nucleus because it frequently fragments or dissolves. It makes little difference what the function of the

[4] Untersuchungen über Bau, Kernteilung und Bewegung der Diatomeen. Aus dem Zoologischen Institut der Universität Heidelberg. Leipzig, 1896.

[5] Über die sogenannten Centralkörper der Zelle und ihre Bedeutung. Verhandl. des Naturhist.-Med. Vereins zu Heidelberg. N. F. 4: 535-538. 1891.

centrosphere is finally discovered to be; the presence of the body must be explained. That its function may have been misinterpreted is no argument against its existence. The centrosome may be a mere insertion point for spindle threads and cytoplasmic radiations, as Heidenhain seems to suppose; it may be the special organ of division and a truly directive sphere; it may be even more, and have some function in transmitting hereditary characteristics; but whatever its function may be, the point to be decided first is its existence. If this is established, questions as to its origin, purpose, and permanency naturally follow.

The appearance of two centrospheres at the poles of the spindle (*figs. 3, 34*), it seems to me, cannot be explained by a crossing of cytoplasmic filaments; by an attraction from the periphery to a common center; or by the rather lately broached idea of a sort of whirlpool in the cytoplasm. So far as the writer is able to judge, no one has attempted to offer a satisfactory explanation of these double centers at the poles on the theory of their temporary nature, since he called attention to them in 1894.[6] At this stage these bodies can be identified readily, and there is no danger of mistaking other granules of the cytoplasm for centrospheres.

In this connection I wish to refer to Humphrey's[7] implied criticism of my former work on centrospheres. He intimates that, having largely used Hermann's method of staining centrospheres, I may have mistaken various proteid granules in the cytoplasm for true centrospheres. My only reply to this is that I was fully aware of the fact that many small bodies in plant cells often greatly interfere with the identification of centrosomes, and, therefore, a large number of stains and methods of killing were used in order to eliminate any faulty observations which might be possible in using only a single method of preparation. It must be recognized that methods of fixing and staining do not give the same results when used by different observers.

[6] The nature and distribution of attraction spheres and centrosomes in vegetable cells. BOT. GAZ. **19**: 449. 1894.

[7] On some constituents of the cell. Ann. Bot. **9**: 574. 1895.

Even plants of the same species and different parts of the same plant, when treated in exactly the same way, will react quite differently, and one must practically invent a new process for every form studied if reliable results are to be obtained.

THE ANTIPODAL REGION.

As already stated, the three antipodals are surrounded at an early stage by very definite cell walls, although in certain cases the formation of walls may be delayed for some time. When the embryo is two-celled, the embryo sac is still quite narrow, and tapers very gradually into the antipodal region (*figs. 36, 37, 39*); but after this it widens out greatly, leaving the antipodal region, which in the meantime has developed very thick cell walls, as a sort of vermiform appendix to the lower part of the sac (*figs. 40, 41, 42, 43*). The antipodal region, with its three nuclei, persists even in the fully formed ovule, where it always produces a very striking appearance because of the constancy with which it preserves its original shape and dimensions. In these late stages, however, the antipodal nuclei stain a very deep color, so that they appear almost entirely homogeneous (*figs. 40, 41, 42, 43, 44, 73*). It is probable that this persistence of the antipodal region may be much more common than is generally supposed, and that it may often have been overlooked and reported as disappearing when it was actually present and persisting even in the mature ovule.

DEVELOPMENT OF THE ENDOSPERM.

In the development of its endosperm Sagittaria presents some very interesting peculiarities. The first division of the definitive nucleus takes place about the same time as the first division of the oospore; and what is most remarkable, at this division a cell plate is formed between the daughter nuclei, which cuts the embryo sac transversely into two compartments (*figs. 36, 37, 38, 39*). This transverse wall will be called the partition wall. Because of the difference in the behavior of these two nuclei, to avoid ambiguity, the one on the micropylar

side of the partition wall will be called the upper endosperm nucleus, and the one on the antipodal side of the partition wall the lower endosperm nucleus.

The upper endosperm nucleus immediately begins to travel upward on the convex side of the embryo sac wall, and immediately begins a rather rapid free nuclear division (*figs. 37, 38, 39*). At the same time the ovule takes on its campylotropous shape, the sac almost doubling on itself, and the elongation practically all taking place above the partition wall (*figs. 38, 41, 73*). In the early stages the free endosperm nuclei are about equally distributed from the embryo down to the partition wall. After the embryo has reached nearly the mature condition the numerous free endosperm cells, which in the meantime have accumulated above the partition wall, begin an active process of free cell wall formation, forming quite a large cap, which extends over the tip of the cotyledon and crowds down upon the partition wall, forcing its outer margin downward (*figs. 44, 73*).

In the meantime no such process has been going on in the compartment below the partition wall. The lower endosperm nucleus does not divide for a long time, but increases considerably in size (*fig. 40*). Its first division usually occurs at the time when the embryo is about seven or eight celled, and it is nearly always divided when the embryo is from nine to eleven celled. Sometimes one of the nuclei may divide again, thus producing three nuclei (*fig. 43*), or it may be that the three nuclei were produced by direct division. No more than three nuclei were observed in any stage, although it is possible that sometimes there may be more. These nuclei increase enormously in size, being as large or even larger than the giant nucleus of the vesicular suspensor cell. They are nearly always closely crowded together (*fig. 43*), and at the time of the free cell wall formation of the upper endosperm they appear to break up and take on the deep stain which is characteristic of the antipodal nuclei (*fig. 44*). When the ovule has reached maturity, all that can be seen of these nuclei is an irregular mass of red stained material situated just above the antipodal region in a sort of pocket

cut off by the partition wall (*fig. 73*). Just what the function of these lower endosperm cells may be the writer is not prepared to state. In the earlier stages the whole compartment has a glandular appearance, much like the vesicular cell of the suspensor. It seems possible, therefore, that it may play an important part in the transfer of food material from the funicular region, beyond the antipodals, to the cotyledon, and especially in facilitating the formation of the cap of endosperm which covers the tip of the cotyledon.

DEVELOPMENT OF THE EMBRYO.

After fertilization the large spherical nucleus of the oospore lies in about the same position as that occupied by the oosphere nucleus before fusion (*fig. 32*). The oospore now begins to push downward into the sac and is surrounded by a definite cell wall, and usually one or more large vacuoles appear above it (*figs. 33, 35*). The formation of vacuoles in the rear of moving nuclei seems to be of quite general occurrence, especially in the embryo sac. The nuclei seem to be carried along by the streaming of the cytoplasm, which as it advances develops a vacuole behind it.

The first division of the oospore is transverse (*figs. 36, 38, 39*). After this the lower cell elongates and divides again by a transverse wall, making the first three cells of the pro-embryo, which are thus produced in acropetal succession (*fig. 46*). The upper cell never divides, and forms the vesicular suspensor cell, which immediately begins to increase enormously in size. The lowest cell gives rise to the terminal cotyledon, and its first division, which may occur immediately (*fig. 47*) or may be delayed for a considerable length of time, is always longitudinal. The middle cell gives rise to the apex of the stem, the hypocotyl, the root tip, and all the suspensor cells except the vesicular cell, its divisions occurring in basipetal succession. The general course of events, therefore, is the same as that given by Hanstein and Famintzin for *Alisma Plantago*, except in certain details and variations which will be mentioned later, and

does not agree with my own observations on the early development of the embryo of Alisma, where I found four cells produced in acropetal succession before the longitudinal division of the terminal cell. I am inclined now to regard this as only an exceptional variation. However, such a variation may also occur in Sagittaria, since the succession of divisions of individual cells in an embryo does not seem to be so invariable as was once supposed. Sometimes as many as five cells in a single chain were observed without any indication of a longitudinal division in the terminal cell (*fig. 52*). In such a case, of course, it is impossible to tell just how the various cells originated unless one is fortunate enough to find cases in which the nuclei are in the spindle stage.

I do not consider it proper in this case to call the terminal cell, which gives rise to the cotyledon, the embryo cell, but shall call it what it really is, the cotyledon cell. Nor does it seem reasonable to include the middle cell in the suspensor. It will be seen that the development of the embryo proceeds gradually, and to call one cell a suspensor cell which at the next division becomes a cell of the embryo, is drawing an arbitrary line where none exists. The cell at the upper or micropylar end can be called properly a suspensor cell, since it never contributes to any part of the embryo proper, but is subsequently destroyed. The cells which finally become a permanent part of the suspensor between the vesicular cell and the embryo are variable in number and are a late development. There is always, except in rare cases, at least one cell between the developing embryo and the vesicular suspensor cell, which by basipetal divisions contributes to the development of the root-tip, and finally develops a filamentous suspensor, and this cell may be called a temporary suspensor cell. But it seems to me that in cases like Sagittaria the only reasonable terminology is to regard as embryo cells all those which go to make up the embryo, and to restrict the term suspensor to that part which never contributes to the formation of the embryo.

Taking the usual course of events, the third division is in

the middle cell, which divides transversely, making a chain of four cells (*figs. 46, 48*). The succeeding division is in the terminal cell, which divides longitudinally, giving rise to the first two cells of the cotyledon (*figs. 49, 50*). The cell *b* (*fig. 49*) gives rise to the stem tip, while the cell *d* (*fig. 49*) divides transversely, forming the six-celled pro-embryo with five tiers of cells (*figs. 51, 53*). The next division is in the cell *b*, which divides longitudinally (*figs. 54, 55*), and gives rise to the seven-celled pro-embryo (*fig. 56*). During this time the remaining synergid is a very active cell, and appears to assist the vesicular cell in its function (*figs. 46, 48, 54, 55*). There now occur several divisions in rapid succession, but not always in the same order. Usually the two terminal cells divide longitudinally; the cell *d* (*fig. 59*) also divides longitudinally; while in the cell above this transverse division occurs, giving rise to an eleven-celled pro-embryo with six tiers of cells (*figs. 57, 58, 59, 60, 61*). Each one of the four terminal cells now divides transversely (*fig. 62*), so that the young cotyledon becomes an octant. That the process is not always so typical will be seen from *fig. 63*, where one of the four cells has divided longitudinally, and another one is dividing transversely, while there are only five tiers of cells.

After the formation of the octant of the cotyledon, the next thing which usually occurs is the cutting off of the dermatogen by periclinal divisions in these eight cells, and the same process usually goes on in the tier above at the same time (*fig. 64*). The cell in tier *e* (*fig. 64*) now divides longitudinally, while the cell *f* (*fig. 64*) still remains single (*fig. 65*). In one case, however, I observed that this cell *f* (*fig. 66*) also had divided longitudinally. This is a very interesting variation, since it would change the entire course of the development of the root tip and suspensor. It is another illustration showing that no hard and fast lines can be drawn for the development of an embryo. The early stages of the embryo are quite apt to show variations which from their fundamental character must change the whole future course of development. The predestination of cells for

a certain fixed course of development agrees neither with our present ideas of development nor with observed facts.

Taking up again the general course of embryonic development, we have next the differentiation of the apical area of the stem, which begins in a hypodermal cell of tier *b* (*fig. 67*). Another transverse division of the cell *f* (*fig. 64*) occurs, while at the same time the cotyledon also undergoes further development (*figs. 67, 68, 69*). It will be noticed, therefore, that the cotyledon is the earliest region to be developed, and that the apex of the stem follows. The development of the apical region of the stem is continued by transverse divisions of the neighboring dermatogen cells of tier *b* (*fig. 69*), and later the remaining cells of this tier also divide by transverse walls (*fig. 70*). At this stage the vesicular suspensor cell appears to be in its most active condition, but from this time on it begins to disorganize. At this time, and for some time later, the entire embryo is meristematic, and division may take place in any part. In the meantime, after considerable growth, the cell *g* (*fig. 70*) divides by a transverse wall, forming another tier *h*, the lower cell dividing again longitudinally into four cells (*fig. 71*). Whether tiers *e* and *f* (*fig. 71*) arise from tier *e* (*fig. 70*) I could not determine, although from the difference in size of the cells of the two tiers it seems probable that they do not. The embryo now begins to elongate, showing a deep depression on the side where the stem apex is situated, and there is a farther development of cells between the embryo and the vesicular cell (*fig. 72*). At this stage the embryo sac is of almost mature proportions, and the embryo as it grows downward bends around the curve of the sac, very likely because of the mechanical resistance offered by the walls within which it is confined, and thus acquires its hooked form (*fig. 73*.)

DIFFERENTIATION OF DERMATOGEN, PERIBLEM, AND PLEROME.

The development of dermatogen begins at the apex of the cotyledon, and as the embryo develops the dermatogen extends farther and farther toward the point where the apex of the root

will finally be developed. Just how many tiers of cells go to make up the hypocotyl and root tip it seems impossible to determine; in some cases, no doubt, more tiers than in others, since the growth upward seems to have no special definite limit. A variable number of suspensor cells are developed. Sometimes as many as six cells are left for the suspensor, besides the vesicular cell, after the definite limits of the embryo are determined by the completion of the dermatogen around the root tip (*fig. 75*). At this stage the suspensor is usually broken. The dermatogen is thus fully developed before the plerome strand is differentiated enough to be recognized. A hypodermal cell of the root tip is differentiated, and by transverse division forms the initial cell of the plerome strand (*figs. 76, 77, 78, 79*), while at the same time the central primary meristematic tissue is developed into the plerome by longitudinal cell divisions. The plerome and periblem in most cases can be traced downward to this initial cell. The calyptrogen is developed by transverse divisions of a small number of dermatogen cells of the root tip, which by further divisions form a very small root cap for the young embryo (*figs. 77, 78, 79*).

The arrangement of tissues in the mature embryo is well shown by cross sections. At the apex a single central cell appears (*fig. 80*), and a little farther up the differentiation of the plerome strand and periblem are well marked out (*fig. 81*). A section about through the center of the hypocotyl shows a well-marked dermatogen, and inside of this three layers of large periblem cells with large intercellular spaces. In the center the plerome is composed of a bundle of twelve or more long narrow cells, surrounded by a circle of nine or more larger cells forming a sheath (*fig. 82*). Finally, a longitudinal section through the stem apex shows a very deep cleft with the first leaflet already somewhat developed (*fig. 83*).

SUMMARY.

1. Broadly speaking, the development of the pollen grain, embryo sac, and embryo of *Sagittaria variabilis* is the same as in

Alisma Plantago, although there are some striking and important differences.

2. The generative nucleus divides into the two sperm nuclei long before the dehiscence of the anther, making a three-nucleated pollen grain.

3. In the eight-celled embryo sac the upper polar nucleus is by far the largest, and the point of contact and of fusion of the two polar nuclei is in the lower part of the sac, the fusion usually being completed before fertilization.

4. During the fusion of the polar nuclei the centrospheres and nucleoli also appear to fuse.

5. The three antipodal cells are usually surrounded by cell walls before fertilization, and the antipodal region, having developed unusually thick walls, retains its original size and contents even when the embryo is fully formed, projecting somewhat like a vermiform appendix beyond the limits of the enlarged sac.

6. After conjugation, the first division of the definitive nucleus takes place at about the time of the division of the oospore, and at this first division a cell plate is formed making a partition wall which completely separates the embryo sac into two parts.

7. The lower endosperm nucleus divides once or twice, forming two or three free nuclei which enlarge enormously and seem to disintegrate when the embryo is mature.

8. The growth and curving of the embryo sac is practically all above the partition wall; and in this part the upper endosperm nucleus forms many small free cells, those aggregated in the lower part, above the partition wall, finally being surrounded by cell walls and forming a sort of cap over the tip of the cotyledon.

9. The pollen tube expands as it enters the embryo sac and passes down on one side past one of the synergids, which disappears at this time.

10. The two sperm nuclei both enter the embryo sac with the pollen tube, but only one leaves the tube and takes part in fertilizing the oosphere.

11. The sperm nucleus is nearly always seen with two very

distinct centrospheres preceding it as it passes through the tip of the tube.

12. When the sperm nucleus passes out of the tube, the apex of the tube appears to soften, and the sperm nucleus, with its centrospheres, appears to break out abruptly, leaving a distinct opening in the tip of the tube, the edges of which often appear ragged; and from this perforation cytoplasm is seen to escape after the sperm nucleus.

13. At the approach of the pollen tube the nucleus of the oosphere is greatly affected, being drawn out into a large bulge toward the approaching male cell. Sometimes two prominent centrospheres appear just on the top of this bulge.

14. Centrospheres appear in resting nuclei and in division stages, and just before the contact and during the fusion of the two sexual nuclei two pairs of centrospheres appear, which seem to fuse simultaneously with the sex nuclei.

15. The remaining synergid persists for a long time above and somewhat to one side of the vesicular suspensor cell, apparently in an active and healthy condition.

16. After fertilization the oospore pushes downward and divides by a transverse wall.

17. The second division of the pro-embryo is in the lower cell, also by a transverse wall. Of the three cells thus developed in acropetal order, the uppermost cell never divides again, but enlarges greatly, forming the vesicular suspensor cell; the lowest develops into the cotyledon, while the middle cell gives rise, by an indefinite number of divisions in basipetal order, to the stem apex, hypocotyl, root tip, and a few suspensor cells.

18. The cell divisions during the formation of the embryo do not occur in regular order, and though the succession of cells follows some general plan, there are frequently remarkable variations which must necessarily change the whole course of development.

19. The cotyledon is first differentiated, and next the stem apex, which develops from a lateral hypodermal cell in the first tier above the terminal cotyledon cell. The hypocotyl develops

from one or two tiers above the stem apex tier, while the root apex develops from an undetermined tier above the hypocotyl region.

20. Beyond the root apex a short suspensor of a single chain of cells, variable in number, connects the embryo with the large vesicular suspensor cell.

21. In the mature embryo the dermatogen, periblem, plerome, and calyptrogen are well differentiated, the plerome strand and periblem cylinder terminating in a single initial cell just within the calyptrogen layer.

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EXPLANATION OF PLATES XX–XXVI

PLATE XX.

FIG. 1. Section of a nectary showing the position of the secreting cells. × 90.

FIG. 2. Pollen mother cells in the mother star stage, with centrospheres at the poles. × 800.

FIG. 3. Pollen mother cell with two centrospheres at each pole. × 800.

FIG. 4. Tetrad stage. × 800.

FIG. 5. Microspore. × 800.

FIG. 6. Pollen grain with two nuclei; the generative nucleus has two centrospheres. × 800.

FIG. 7. Pollen grain with the generative nucleus divided into the two sperm nuclei. × 800.

FIGS. 8 and 9. Mature pollen grains. × 800.

FIG. 10. Embryo sac with four nuclei. × 800.

FIG. 11. Mature eight-celled embryo sac; the antipodals are already surrounded by definite cell walls. × 800.

PLATE XXI.

FIG. 12. Outline sketch of mature eight-celled embryo sac, showing relative size of the nuclei. × 600.

FIG. 13. Conjugating polar nuclei, showing one pair of centrospheres. × 600.

FIG. 14. Embryo sac with the polar nuclei partly fused; the antipodal cells are without walls. × 600.

FIG. 15. Definitive nucleus nearly complete, with a pair of fused centrospheres on opposite sides; the two nucleoli are still distinct. × 600.

FIG. 16. Definitive nucleus with three nucleoli distinct, and two centrospheres on opposite sides. × 600.

FIG. 17. Definitive nucleus with two large nucleoli apparently fusing. × 600.

FIG. 18. Definitive nucleus with one small nucleolus and two large fusing nucleoli. × 600.

FIG. 19. Embryo sac showing one synergid, the oospore, the definitive nucleus, and two antipodals; the definitive nucleus has but one large nucleolus. × 600.

FIG. 20. Upper end of an embryo sac, showing the arrangement of the egg apparatus. × 600.

FIG. 21. Upper end of embryo sac with pollen tube entering; the oosphere nucleus with two prominent centrospheres. × 600.

FIG. 22. Upper end of an embryo sac with pollen tube; one sperm nucleus in the tip of the tube preceded by two centrospheres. × 600.

FIG. 23. Pollen tube with a sperm nucleus at the tip preceded by two centrospheres. × 600.

PLATE XXII.

FIG. 24. Upper end of embryo sac with pollen tube; two sperm nuclei in the tube, the one at the tip preceded by two centrospheres. × 600.

FIG. 25. Embryo sac with definitive nucleus, one antipodal, one synergid, oosphere, and pollen tube with two sperm nuclei; several granules between the oosphere nucleus and the sperm nucleus at the tip of the tube. × 600.

FIG. 26. Upper end of embryo sac with pollen tube. × 600.

FIG. 27. Upper end of embryo sac; the lower sperm nucleus is just leaving the tube, and between it and the oosphere is a mass of granular protoplasm. × 600.

FIG. 28. Upper end of embryo sac; the sperm nucleus with prominent centrospheres has left the tube and is approaching the oosphere. × 600.

FIG. 29. Part of an embryo sac with definitive nucleus, oosphere, and pollen tube; the oosphere nucleus is greatly bulged out on the side toward the sperm nucleus; sperm nucleus just leaving the tube. × 600.

FIG. 30. Upper end of embryo sac; the sperm nucleus has just left the pollen tube, which shows a perforation at the tip from which protoplasm is escaping; four very prominent centrospheres appear at the two angles of the approaching sexual nuclei. × 1125.

FIG. 31. A little later stage than *fig. 30;* the sexual nuclei are in contact, and two pairs of centrospheres appear above and below; one of the synergids lies in front of the tube, the other has disappeared. × 1125.

FIG. 32. Upper end of embryo sac after fertilization, with large spherical oospore nucleus, and pollen tube containing the remaining sperm nucleus. × 1125.

FIG. 33. Oospore beginning to descend; the pollen tube is beginning to disappear; above the oospore lies the remaining synergid. × 600.

FIG. 34. A cell from the tip of a young embryo with nucleus in the daughter skein stage: two large centrospheres at each pole. × 850.

PLATE XXIII.

FIG. 35. Upper end of embryo sac with oospore and synergid. × 400.

FIG. 36. Embryo sac with a two-celled pro-embryo; two endosperm nuclei separated by a distinct cell wall stretching across the sac, and three antipodal cells. × 400.

FIG. 37. Lower end of embryo sac, a little later than *fig. 36*, showing the position of the first two endosperm cells and the cell wall between them. × 400.

FIG. 38. Embryo sac with a two-celled pro-embryo and two endosperm cells separated by a cell wall. × 400.

FIG. 39. About the same stage as *fig. 38;* the upper endosperm nucleus is dividing. × 260.

FIG. 40. Lower end of an embryo sac which contains a nine-celled pro-embryo; the upper endosperm nucleus has divided into many free cells while the lower remains undivided; at the base two antipodals. × 400.

FIG. 41. Complete embryo sac with a nine-celled pro-embryo containing a number of comparatively small free endosperm nuclei; the endosperm nucleus below the partition wall has divided into two nuclei which have greatly enlarged; two antipodals appear at the lower end of the sac. × 66.

FIG. 42. Lower end of an embryo sac containing a ten-celled pro-embryo; the lower endosperm nucleus has divided into two; the antipodal region with very thick walls retaining its original size and contour. × 216.

FIG. 43. Lower end of an embryo sac in which the lower endosperm nucleus has divided into three. × 400.

FIG. 44. Lower end of an embryo sac with embryo at the stage represented in *fig. 72;* the antipodal region is still distinct and contains the three original nuclei; the lower endosperm nucleus has remained undivided; above the partition wall the sac is filled for some distance with endosperm tissue produced by the upper endosperm nucleus. × 216.

FIG. 45. Secreting cells from a nectary showing the appearance of the cytoplasm and nuclei. × 400.

PLATE XXIV.

FIG. 46. Three-celled pro-embryo with synergid (*s*) on the side of the vesicular cell *a*; middle cell (*b*) dividing. × 400.

FIG. 47. Three-celled pro-embryo with terminal cell dividing. × 400.

FIG. 48. Four-celled pro-embryo with synergid (*s*) beside the vesicular cell; the free nucleus is endosperm. × 400.

FIG. 49. Four-celled pro-embryo with terminal cell (*c*) dividing. × 400.

FIG. 50. Five-celled pro-embryo. × 400.

FIG. 51. Five-celled pro-embryo with the two middle cells dividing. × 400.

FIG. 52. Five-celled pro-embryo with the cells in a single row. × 400.

FIG. 53. Six-celled pro-embryo with synergid above the vesicular cell. × 216.

FIG. 54. Six-celled pro-embryo with synergid. × 400.

FIG. 55. Seven-celled pro-embryo with synergid above the vesicular cell; the two lowest cells each with a cell beneath, which does not appear in the figure. × 400.

FIG. 56. Seven-celled pro-embryo; the free nuclei are endosperm. × 216.

FIG. 57. Seven-celled pro-embryo with one of the cells at the tip dividing. × 400.

FIG. 58. Upper end of embryo sac with a nine-celled pro-embryo; the nucleus above the vesicular cell belongs to the synergid. × 216.

FIG. 59. Ten-celled pro-embryo. × 400.

FIG. 60. Ten-celled pro-embryo; the two cells not shown belong to lowest tier. × 216.

FIG. 61. Eleven-celled pro-embryo with synergid. × 400.

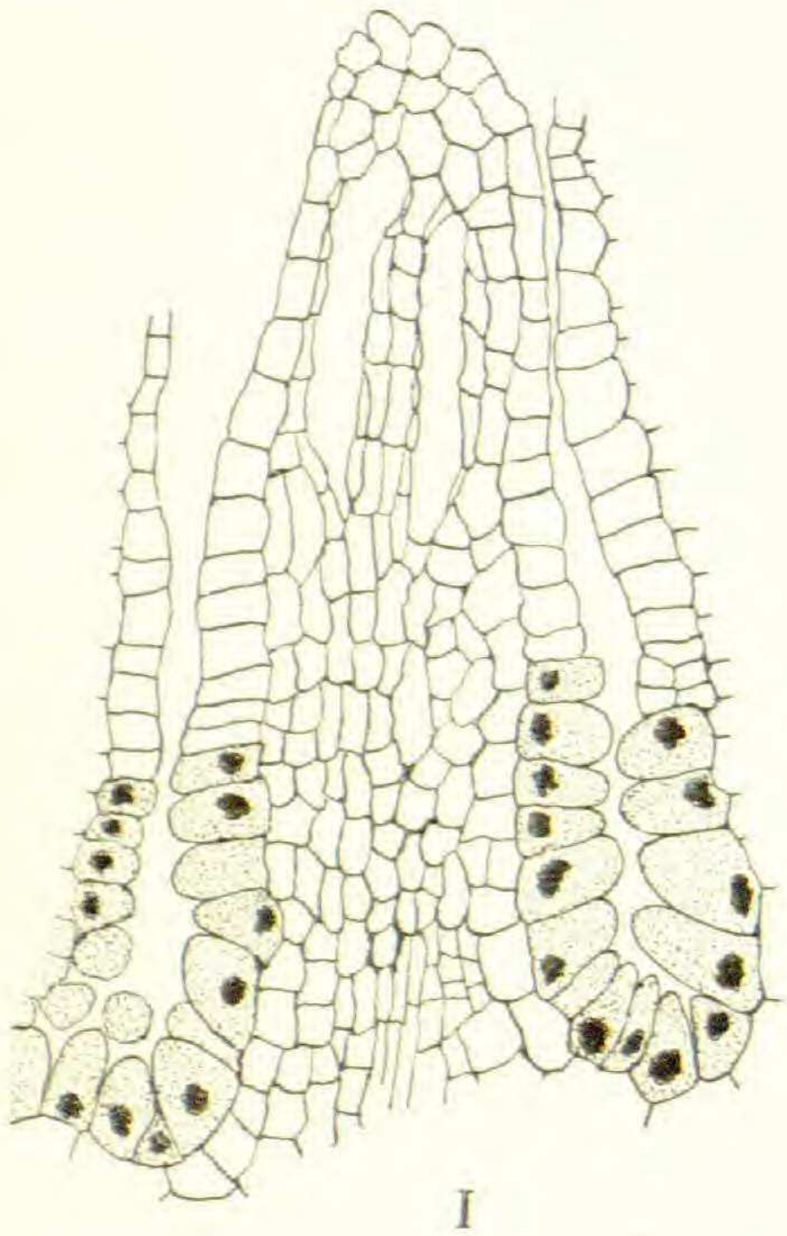
PLATE XXV.

FIG. 62. Twelve-celled pro-embryo with synergid; two cells in the lowest tier not shown. × 400.

FIG. 63. Eleven-celled pro-embryo; the two cells not shown belong to lowest tier. × 400.

FIG. 64. Embryo with dermatogen cut off from the octant which forms the cotyledon. × 260.

FIG. 65. Embryo showing the appearance of the synergid and vesicular cell at this stage, and further development of the dermatogen. × 400.



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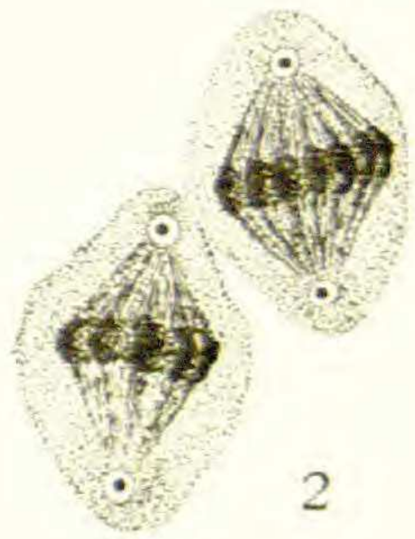

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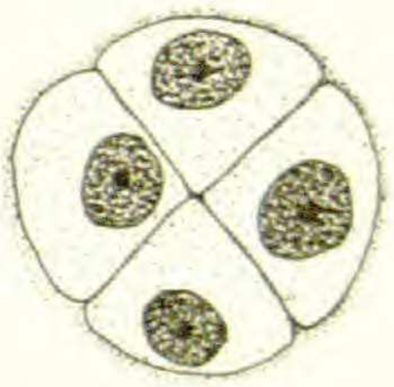

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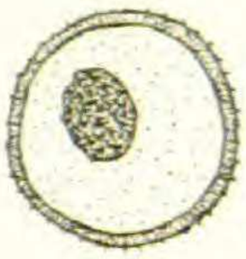

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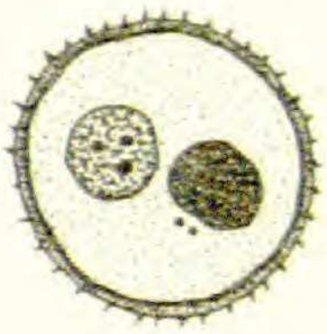

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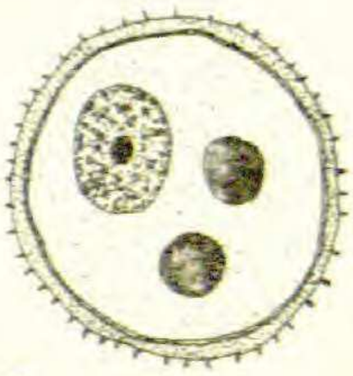

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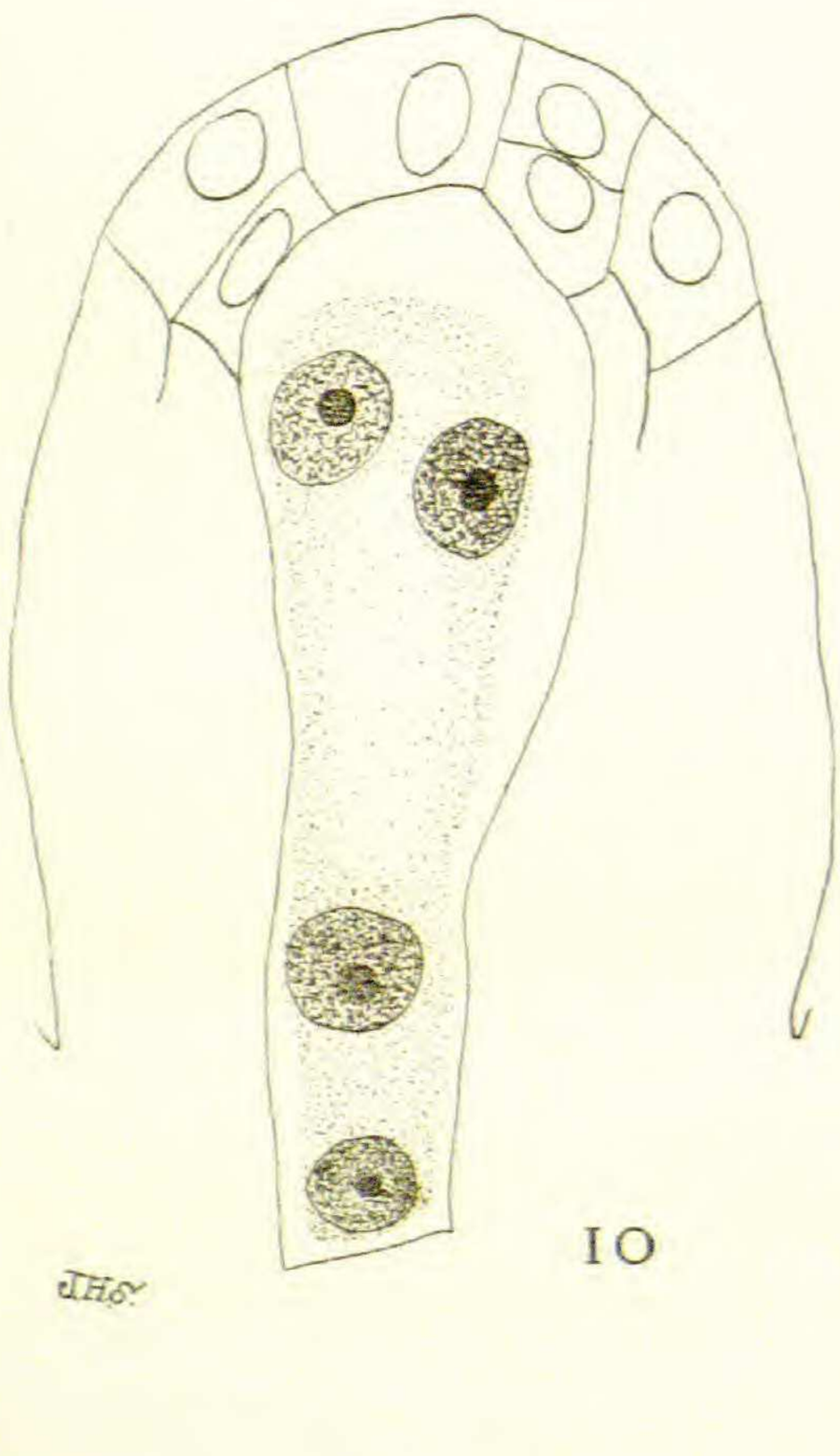

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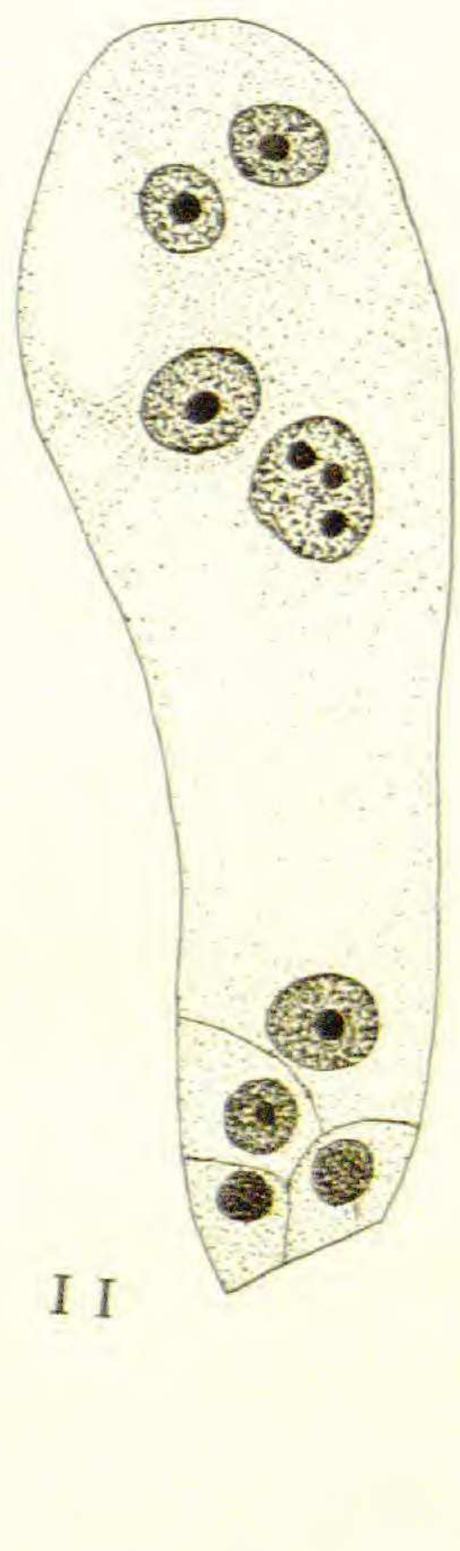

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SCHAFFNER on SAGITTARIA VARIABILIS.